U.S. Application No. 08/447,820 Examiner: M. Woodward

Filed: May 23, 1995 Group Art Unit: 1815

SCHEDULE B2 CLAIM AMENDMENTS

^£ Claim 1, line 6, change "presented" to
-- present --;

Mc line 30, change "to" to -- of --.

Please add the following new claims:

9. A method for detecting a plurality of analytes in a liquid sample of volume V liters, comprising:

loading a plurality of different binding agents, each being capable of reversibly binding an analyte which is or may be present in the liquid sample and is specific for said analyte as compared to the other components of the liquid sample, onto a support means at a plurality of spaced apart small spots such that each spot has a high coating density of one of said binding agents but not more than 0.1 V/K moles of binding agent are present on any spot, where K liters/moles is the affinity constant of said binding agent for said analyte;

contacting the loaded support means with the liquid sample to be analyzed, such that each of the spots is contacted in the same step with said liquid sample, the amount of liquid used in said sample being such that only an insignificant proportion of any analyte present in said liquid sample becomes bound to said binding agent specific for said analyte;

contacting the support with a site-recognition reagent specific for each binding agent in a competitive or non-competitive technique, the site-recognition reagent being capable of recognizing either the unfilled binding sites or the filled binding sites on said binding agent, said site-recognition reagent being labelled with a marker; and





measuring the signal from the marker of the site-recognition reagent in a particular location to detect the presence of said plurality of analytes in said sample.

- 10. A method as claimed in claim 9, wherein each of said spots has a size of less than $1\ mm^2$.
- 11. A method as claimed in claim 10, wherein each of said spots contains more than 10^4 molecules of binding agent.
- 12. A method as claimed in claim 11, wherein each of said spots has less than 0.01 V/K moles of binding agent.
- 13. A method as claimed in claim 11, wherein said binding agents used have affinity constants for said analytes of from 10^8 to 10^{13} liters per mole.
- 14. A method as claimed in claim 11, wherein said binding agents used have affinity constants for said analytes of the order of 10^{10} to 10^{11} liters per mole.
- 15. A method as claimed in claim 11, wherein the volume of said liquid sample is not more than 0.1 liter.
- 16. A method as claimed in claim 11, wherein the volume of said liquid sample is 400 to 1000 microliters.
- 17. A method as claimed in claim 9, wherein said binding agents loaded onto said support means are antibodies for the analytes whose concentrations are to be determined.



